IMPROVEMENT OF H$_2$O$_2$ STABILIZATION IN RAINWATER SAMPLES

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ABSTRACT
Peroxides collected in rainwater readily decompose in the natural matrix, consequently it is hard to estimate their values present during precipitation events. For this reason, only few data concerning the concentration of aqueous H$_2$O$_2$ in dew, cloud and rainwater are available. Therefore, it is necessary to establish a sampling protocol under conditions that assure the real-time estimation of hydrogen peroxide in rainwater samples. Hydrogen Peroxide was determined by a fluorometric method in rainwater collected in fives sites in Mexico. Measured levels for all sampled sites were in agreement with values reported by other authors for marine and continental sites. To establish a sampling protocol, several methods reported for preservation of H$_2$O$_2$ in rainwater were assessed. Sampling protocol proposed in this research involves forming fluorescent dimmer on-site immediately after sample collection, and storing at dark and 4 ºC. This procedure gives a 10 days margin to perform sample analysis, guarantying a minimal decomposition of H$_2$O$_2$ in rainwater samples (approximately 10%) and allowing more time to perform sampling campaigns even in remote sites. The other methods evaluated (freezing, filtering, using acidified samples, adding sodium stannate, and storing samples and their fluorescent dimmer at 0ºC) do not exert any preservation effect over H$_2$O$_2$ levels in rainwater samples.

KEYWORDS: Hydrogen peroxide, Peroxide preservation, Peroxide decomposition, Rain, Peroxide stabilization, peroxide fluorescent detection.

INTRODUCTION
The contribution of NO$_3$ and SO$_4^{2-}$ to acidity of rainwater has become one of the main environmental problems, and it has been subject of numerous researches (Pierson and Chang, 1986; O’Sullivan, 1985; Hansen and Hidy, 1982; Beilke, 1983; Dikaiakos et al., 1990; Bravo et al., 2000; Ceron et al., 2002). Reasons to use SO$_4^{2-}$ ion as the main indicator of acidity, are: 1) its conservative character, 2) there is no a variation in its levels among remote sites, and 3) do not promote acidification in a short time period as
the case of NO₃; (Báez and Belmont, 1987; Báez et al., 1993; Báez et al., 1997a; Báez et al., 1997b; Bravo et al., 2000; Cerón et al., 2002).

There are several oxidants in the atmosphere that play an important role in the conversion of SO₂ to H₂SO₄. In aqueous phase, there are two main routes for SO₂ oxidation: if rainwater pH is greater than 5, O₃ acts as the principal oxidant. On the other hand, if rainwater pH is minor than 4.5, H₂O₂ contributes in a great proportion to the acidification process (Zika et al., 1982; Chameides, 1984; Calvert et al., 1985; Sakugawa et al., 1993; Sauer et al., 1997). H₂O₂ is very important in the oxidation process of S(IV) in aqueous phase, then it is necessary to characterize its levels present in air, cloud water and rainwater. Specifically, measurement of hydrogen peroxide concentrations in water droplets can afford an estimation of the gas phase concentration and oxidation rate of sulphur dioxide.

Until now, not many information on the concentration of atmospheric aqueous H₂O₂ is available, where several interferences associated with the sampling procedure for H₂O₂ in gaseous phase have been reported (Cohen et al., 1967; Possanzini et al., 1988; Sakugawa and Kaplan, 1993). However, some works about determination of H₂O₂ content in rain and cloud water (Kok, 1980; Zika et al., 1982; Cooper et al., 1987; Sakugawa and Kaplan, 1987; Miller and Kester, 1994; Willey et al., 1996; Sauer et al., 1997; Deng and Zuo, 1999; Yuan and Shiller, 2000; Ortiz et al., 2000), report H₂O₂ concentrations from 1 to 135 µM. Unfortunately, H₂O₂ present into water droplets readily decompose by a catalytic process that involves a great variety of metals (Kremer, 1985; Greadel et al., 1986; Luňak and Sedlak, 1992; Zepp, 1992; Sedlak et al., 1997). Ortiz et al. (2000) showed that substantial decomposition of H₂O₂ levels in rainwater and cloud water samples take place during the time elapsed between collection and analysis, time values reported fluctuate from 80 to 700 min. For this reason, in most of methods proposed for hydrogen peroxide analysis in rainwater, samples must be analyzed immediately after its collection, making very hard to estimate H₂O₂ levels that existed in the sampling moment. Due to the scarce measurements of H₂O₂ in rainwater and the lack of confidence in gas phase measurements, most of the results reported have only a qualitative character until now, and it has no been possible to find defined correlations or a tendency between data.

Hence, in order to assess quantitatively the process that control H₂O₂ atmospheric levels, more measurements of H₂O₂ in rainwater are required, specially, under a great variety of preservation conditions that minimize its rate of decomposition after collection and assure its proper determination. On the other hand, some authors report that using fluorometric methods samples can be preserved during several days (Sakugawa and Kaplan, 1987; Sauer et al., 1997), unfortunately, the conditions and the preservation time have not been well established.

As a result, the objective of this work was to determine H₂O₂ levels in rainwater, using different preservation methods, compare the results obtained in each one, and to establish a sampling protocol that assure its quantitative determination, even in remote sites. This result will permit in a subsequent stage of this research, to assess quantitatively the processes that control atmospheric H₂O₂ levels in gas and aqueous phase in marine, coastal and continental sites.

**EXPERIMENTAL METHOD**

**Analytical Technique**

A derivatization technique has been reported by several authors for the combined determination of hydrogen peroxide and some organic hydroperoxides in precipitation samples (Keuken et al., 1988; Sakugawa and Kaplan, 1987; Olszyna et al., 1988; Kelly et al., 1985; Zika et al., 1982; Lazrus et al., 1985). This analytical technique combines the sensitivity of fluorometry with the specificity of enzymatic reactions. This fluorometric method allows the sensitive detection of hydrogen peroxide and organic peroxides in aqueous phase, through enzymatic catalysis and formation of fluorescent species, followed by the speciation of H₂O₂ and organic peroxides through a selective decomposition of H₂O₂ that is catalyzed by catalase enzyme.

The analytical procedure is based on the fluorescent detection of the dimmer obtained from the reaction between p-hydroxyphenylacetic acid and H₂O₂ at pH 8.5, in the presence of peroxidase "horseradish". The dimmer is quantified by fluorescent detection using an excitation wavelength of 320 nm and measuring the emission intensity at 400 nm. This fluorescence is therefore, proportional to the H₂O₂ concentration. The specific detection is
performed through the destruction of H₂O₂ by catalase enzyme, and the difference between values obtained with (organic peroxides) and without catalase (total peroxides) gives the total H₂O₂ content (Lazrus et al., 1985; Lazrus et al., 1986; Kok et al., 1986; Sakugawa and Kaplan, 1987).

A Spectra-system FL-3000 detector connected to an HPLC (high performance liquid chromatography) equipment with a single channel was used to measure hydrogen peroxide levels. Sample was loaded into a sample injection valve using a glass syringe with a Teflon plunger, injection system was modified to ensure that sample was injected directly to detector. Specific detection of H₂O₂ levels in a single channel detector involves manual addition of catalase and the analysis of two aliquots, thereby, more sample, chemical reagent and analysis time are required. Blank samples were made using pure water both with and without addition of catalase. The fluorescence for each blank value was subtracted from each sample value.

Two aliquots of 1.8 ml of sample were required. First sample aliquot or standard was transferred with a pipette to a 10 ml vial, then 0.3 ml of 0.1 M potassium hydrogen phthalate (pH 5.5) and EDTA (1x10⁻³ M) were added; Fluorescent reagent [0.3 ml of 1x10⁻² M of p-hydroxyphenylacetic acid (Aldrich) and peroxidase (0.8 mg of 10 purpuro-gallin units ml⁻¹ (Sigma))] were added to it. After 1 minute of reaction time, 0.3 ml of 0.2 M NaOH was added to stabilize the generated fluorescence. This procedure allows the determination of total peroxide (H₂O₂ and organic peroxide).

To discriminate H₂O₂ from organic peroxides, 0.3 ml of catalase reagent (500 unit ml⁻¹, Sigma) was added to the second sample aliquot (1.8 ml). After 40 seconds, fluorescent reagent with peroxidase (0.3 ml) was added. Finally, fluorescent reaction obtained was stabilized by adding NaOH 0.2 M. H₂O₂ is more rapidly decomposed by catalase than organic peroxides, and the difference in fluorescent signals obtained in both cases, with and without catalase, represents the total content of H₂O₂ in the rainwater sample or standard solution.

H₂O₂ standards were prepared by dilution of a stock H₂O₂ standard. H₂O₂ concentration of the stock standard solution was determined by titration with KMnO₄. A 10,000 µM H₂O₂ standard was prepared by dilution of commercially available 30% H₂O₂. Stock H₂O₂ solutions prepared in this manner are found to decay to a rate about 1% per month. Working standards were prepared daily. Glassware to be used in preparing H₂O₂ standards should be washed with soap, rinsed, and allowed to soak in deionised water for several days with frequent water changes. In analytical work with H₂O₂ it is important that all solutions be prepared from water free of bacteria as well as ionic impurities. In this work, solutions were prepared with purified water by deionization cartridges and stored at dark and 4ºC. Another important detail of the analysis procedure is to use freshly made fluorescent reagent (p-hydroxyphenylacetic acid + peroxidase enzyme). This reagent decomposes after some storing time, consequently results are altered and precision is lost. Differences in duplicate measurements were below 10%.

Concentrations above 15 µM H₂O₂ saturated photomultiplier tube, due to detector sensitivity, thus it was not possible to measure H₂O₂ levels directly. For that reason, in order to extend the calibration curve, it was indispensable to make dilutions. Effectiveness of this procedure was tested measuring 1:2, 1:5 and 1:10 dilutions of standard solutions and samples. Results showed that the best fitting is obtained with 1:10 dilution of fluorescent dimmer, consequently, reagents are saved and calibration curve is extended, allowing detection from 0.5 to 100 µM of H₂O₂ in rainwater. Detection limit of this analytical method was 0.5 µM of H₂O₂.

Sample Collection
Rainwater samples were collected in five sites in Mexico. Figure 1 comprises a map showing their geographical location: site one was positioned on the roof of the Atmospheric Sciences Centre Building in Mexico City; site two was placed on the roof of a residential site in Mexico City; site three was sited in a residential area in Orizaba City; site four was located at the top of a meteorological station located in Puerto Morelos Town; and site number five was positioned in Tropical Pacific Ocean on board of Oceanographic Ship “PUMA”. In all cases, rainwater was collected using a 2 liters glass beaker, and the collectors were far from any possible emission source. Samples were transferred to glass bottles protected from light.
RESULTS AND DISCUSSION

A study with standard solutions and samples was performed to observe the decay of H$_2$O$_2$ levels after their preparation or collection, using different methods to preserve them. The methods evaluated to preserve H$_2$O$_2$ levels in rainwater samples were:

a) Freezing rainwater samples.
b) Filtering rainwater samples.
c) Using acidified rainwater samplers.
d) Adding sodium stannate to rainwater samples.
e) Storing samples and fluorescent dimmer at 0 and at 4°C.

In all cases, it was observed that total peroxides were constituted by hydrogen peroxide, and organic peroxides fraction was not significant. On the other hand, the values obtained were of the same order of magnitude of marine, continental and coastal sites values reported by several authors (Kok, 1980; Zika et al., 1982; Cooper et al., 1987; Sakugawa and Kaplan, 1987; Miller and Kester, 1994; Willey et al., 1996; Sauer et al., 1997; Deng and Zuo, 1999; Yuan and Shiller, 2000). Some works (Kok, 1980; Deng and Zuo, 1999) suggest that storing samples at 0°C, makes possible to preserve them for H$_2$O$_2$ analysis. However, Sauer et al. (1997) found in samples stored at −18°C, that hydrogen peroxide levels decrease 50%, after 6 hours of their collection.

From August 27 to September 27 of 2000, 43 samples were obtained at Puerto Morelos Town and preserved only by freezing. Results show an average H$_2$O$_2$ concentration of 7.7 µM, ranging from 0.5 to 28.13 µM and are agreement with hydrogen peroxide levels reported for coastal sites; nevertheless analysis of H$_2$O$_2$ levels with time, in samples with a high H$_2$O$_2$ content, reveals that H$_2$O$_2$ levels present in rainwater samples decompose readily during the period comprised between collection and analysis, so it was no possible to determine its levels in a suitable way.

On the other hand, 56 samples collected on the roof of the Atmospheric Sciences Centre Building in Mexico City, from April 2 to October 6 of 2001, were preserved only by freezing; in this case, it was found that H$_2$O$_2$ levels show a pronounced decay in both standard solutions and samples, therefore there was not any observed preservation effect over samples.
Results showed an average $\text{H}_2\text{O}_2$ concentration of 15.24 $\mu$M, ranging from 1.83 to 59.46 $\mu$M. Typical decay curves in both cases (Puerto Morelos Town and Mexico City) are shown in Figure 2. In these samples, a rather fast initial decay was observed, followed by a considerably...
slower decomposition until an almost constant value is reached (or a plateau value). As a result, it was necessary to study the decay of H$_2$O$_2$ levels using different preservation methods, before the next sampling period. Deng and Zuo (1999) established that H$_2$O$_2$ levels do not decrease with time in filtered samples; however, 55 samples collected on the roof of a residential site in Mexico City from March 24 to October 6 of 2001 were preserved by filtering, and it was observed that filtering does not exert any preservation effect over H$_2$O$_2$ levels of samples and standard solutions (Figure 3). Results show an average H$_2$O$_2$ concentration of 20.88 µM, ranging from 2.55 to 71.2 µM.

In addition, Ortíz et al. (2000) found that more than 70% of the peroxides collected in acidified rainwater samplers (pH<2) are present after 40 hours of their collection, at dark and ambient temperature. While, Sauer et al. (1997) proposed to use sodium stannate (J.T. Baker) to preserve the samples for H$_2$O$_2$ determination. 44 samples collected in a residential site in Orizaba City from June 8 to October 27 of 2001 were preserved adding nitric acid to obtain a pH of 2, and adding sodium stannate (J.T. Baker). Results showed an average H$_2$O$_2$ concentration of 18.6 µM, ranging from 1.73 to 86.9 µM. Figure 3 shows that neither nitric acid nor sodium stannate exert any preservation effect over hydrogen peroxide levels present in rainwater samples.

Several authors (Keuken et al., 1988; Olszyna et al., 1988; Kelly et al., 1985) report that in remote sites, samples stabilized with p-hydroxyphenylacetic acid, can be conserved during several days at ambient conditions. In 35 samples obtained at Atmospheric Sciences Centre Building, in Mexico City from August 3 to October 6 of 2001, it was observed that fluorescent dimmer stays stable until 5 or 6 days after its formation; after 6th day, decay is higher in samples than standard solutions (Figure 3). Results show an average H$_2$O$_2$ concentration of 19.24 µM, ranging from 2.37 to 77.92 µM. Similar decay patterns were observed in all samples collected. One additional method assessed was storing samples and its fluorescent dimmer in a cool site at 4°C or freezing at 0°C.

Figure 4 shows a comparison of hydrogen peroxide decay
content in rainwater using cooling or freezing of fluorescent dimmer as sample preservation method. It can be observed that freezing both sample and fluorescent dimmer do not preserve hydrogen peroxide levels. Results show an average H$_2$O$_2$ concentration of 15.46 µM, ranging from 1.51 to 82.7 µM. On the other hand, storing at 4°C does not exert a preservation effect on samples, but assure a minimal decrease in H$_2$O$_2$ levels in the case of fluorescent dimmer, giving a time period of 10 days after collection for their determination by fluorescent analysis.

An additional test was performed in order to determine the minimum collection time between fractions from same precipitation event, and to assure the minimal possible degradation in H$_2$O$_2$ levels present in rainwater. Test consisted of forming the fluorescent dimmer at different times after collection. Results show a variation of 11, 19.8 and 46.4 %, if dimmer is formed at 0.5, 1 and 4 hours after collection sample. Therefore, to assure a variation less than 10%, during sequential samplings, rainwater fractions must be taken each 15 minutes, forming fluorescent dimmer immediately and storing it at dark and 4 ºC.

**CONCLUSIONS**

We propose a sampling protocol for H$_2$O$_2$ levels in rainwater, by taking rainwater samples each 15 minutes during sequential sampling for an individual rain event, forming fluorescent dimmer on-site immediately after sample collection, and storing at 4°C and dark. This procedure, give us a margin of 10 days to perform sample analysis, guarantying a minimal decomposition of H$_2$O$_2$ in rainwater samples (approximately 10%) and permitting to perform sampling campaigns even in remote sites. The other methods evaluated do not exert any preservation effect over H$_2$O$_2$ levels in rainwater samples.

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